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Anti-schistosome chemotherapy enhanced by antibodies specific for a parasite esterase

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SUMMARY

The immune-dependent action of praziquantel has been investigated in *Schistosoma mansoni*-infected mice by passive transfer of rabbit antisera simultaneously with drug treatment. Significant synergistic activity was obtained with polyspecific sera against culture medium extracts of adult worms, but not with sera against detergent extracts or whole worm homogenates. Serum from a rabbit 'infected' with unattenuated *S. mansoni* cercariae was also synergistically active with praziquantel, and from this serum were derived two further active and monospecific sera which immunoprecipitated a 27,000 MW antigen with non-specific esterolytic enzyme activity. The antigen against which the monospecific sera reacted was detected by indirect immunofluorescence on the tubercles of drug-treated worms, but not on control worms. The immune-dependence of praziquantel thus appears related to drug-induced damage on the surface on the worm, which results in exposure of antigens sensitive to damage by antibody.

INTRODUCTION

Drugs are the mainstay of treatment of most infectious diseases, including schistosomiasis, but it has long been debated whether drugs act alone or in concert with the immune response (Ehrlich, 1909). The immune status of the host has been shown recently to affect the outcome of treatment of a variety of experimental parasitic infections such as rodent malaria (Lwin, Targett & Doenhoff, 1987), onchocerciasis (Bianco *et al.*, 1986) and schistosomiasis (Doenhoff & Bain, 1978). With respect to *Schistosoma mansoni* infections in mice, the therapeutic actions of at least four drugs were immune-dependent (Sabah *et al.*, 1987), including that of praziquantel (Gonnert & Andrews, 1977), a relatively new agent that is in increasing use for treatment of human infection. The cure rate achieved with praziquantel in *S. mansoni*-infected mice can be enhanced by transfer of antibodies, either in homologous serum from infected mice (Brindley & Sher, 1987) or in serum from rabbits immunized with antigens released by intact *S. mansoni* worms in culture medium (Doenhoff *et al.*, 1987).

Mouse infection sera and antisera raised against culture extracts contain antibodies against numerous schistosome antigens, thus making it difficult to determine which particular antibody specificity is most relevant to enhancement of drug action. The problem was eased by finding that serum from a rabbit that had been subjected to infection with unattenuated *S. mansoni* cercariae was also synergistically active with praziquantel, while its precipitating antibody activity against adult *S. mansoni* worm antigens was relatively uncomplicated. Monospecific antisera raised against one worm antigen displayed in immunoelectrophoresis by the infected rabbit serum also enhanced the schistosomicidal activity of praziquantel, and the immunoprecipitated antigen in question hydrolysed beta-napthyl acetate, a non-specific substrate of esterases. Indirect immunofluorescence with the monospecific sera indicated that the antigen became revealed on the adult worm surface after *in vivo* drug treatment.

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MATERIALS AND METHODS

Parasite and parasitology

A Puerto Rican strain of *S. mansoni* was maintained by passage in laboratory reared *Biomphalaria glabrata* snails and random bred T.O. strain mice (Tack & Sons, Battlebridge). CBA/Lac strain mice, bred on site, were used as experimental animals. Percutaneous infections of 200 *S. mansoni* cercariae and portal perfusions to estimate worm burdens were performed as described previously (Smithers & Terry, 1965). Untreated control mice (Group a, Table 1) were perfused 42 days after infection; drug +/− serum-treated mice (Groups b, c and d) were perfused 56 days after infection. Percentage reduction in worm numbers in treated Groups b, c and d was estimated relative to number of worms in untreated Group a.

Drug and drug treatment

Praziquantel [2-cyclohexylcarbonyl-1,2,3,6,7,11b-hexahydro-4H-pyrazino(2,1a)isoquinolin-4-one] (Gonnert & Andrews,

Table 1. Test for synergistic action of rabbit antisera with praziquantel in *S. mansoni*-infected mice

Exp.	Group	No. of mice	Praziquantel	Rabbit serum	Mean no. of worms	% reduction
1	a	6	–	—	94±39	
	b	8	+	—	26±13	72
	c	8	+	1002M	33±16	65
2	a	8	–	—	81±11	
	b	8	+	—	41±13	49
	c	8	+	1018S	31±22	62
3	a	8	–	—	89±25	
	b	7	+	—	49±17	45
	c	8	+	993S	27±10	70**
	d	8	+	993T	30±17	66**
4	a	6	–	—	80±10	
	b	7	+	—	38±9	51
	c	8	+	942T	24±7	70**
5	a	8	–	—	57±21	
	b	8	+	—	38±11	33
	c	4	+	1018J	17±19	79*
	d	8	+	1018K	1±1	98***

Sera which caused a significant reduction in worm count relative to that given by drug alone are asterisked (Student's *t*-test; * $P<0.05$, ** $P<0.01$, *** $P<0.001$).

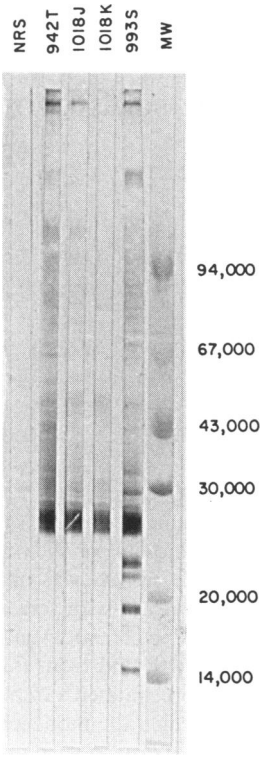

Figure 2. Immunoblot analysis. NRS, normal rabbit serum; MW, molecular weight markers. Numbered sera as in text.

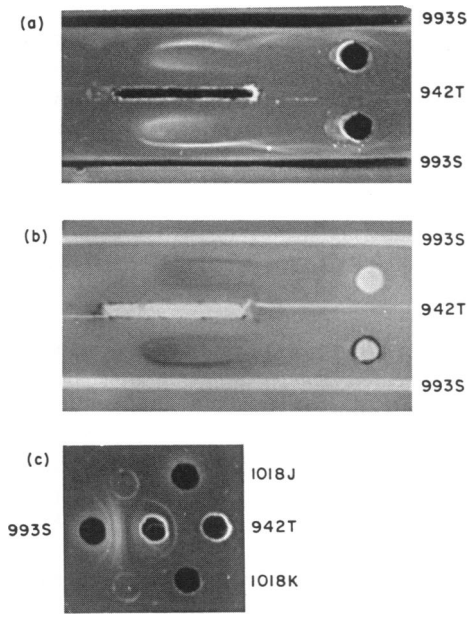

Figure 1. Immunoprecipitation of *S. mansoni* worm antigens by rabbit antisera. (a) Immunoelectrophoresis of deoxycholate extract of adult worms with rabbit sera, photographed over indirect light. (b) Same as (a) over direct light after esterase zymography. (c) Immunodiffusion of culture medium extract of worms with rabbit sera, indicating identity of antigen precipitated by monospecific and infection sera.

1977) was administered to mice in Groups b, c and d orally on Days 35 and 37 after infection at a subcurative dose of 150 mg/kg body weight.

Rabbit antisera

Serum 1002M was produced by injecting a rabbit with unfractionated homogenate of adult *S. mansoni* worms. Serum 1018S was from a rabbit immunized with the antigens released by intact adult *S. mansoni* worms gently agitated for 4 hr in an equal volume of 2% deoxycholate detergent in saline. Sera 993S and 993T were raised by immunizing rabbits with antigens released by *S. mansoni* worms agitated in an equal volume of culture medium 199 for 3 hr at room temperature (Doenhoff *et al.*, 1987). All three worm antigen solutions/suspensions were injected in multiple subcutaneous sites after emulsification in complete Freund's adjuvant. The rabbits were given 1 ml of emulsion/week for eight successive weeks, each injection containing approximately 5 mg (bovine serum albumin equivalent) protein.

Serum 942T was from a rabbit given five percutaneous infections via the ear at 2-week intervals, each infection consisting of approximately 15×10^3 *S. mansoni* cercariae. Sera 1018J and 1018K were from rabbits that had been immunized by adaptation of previously described methods (Goudie, Horne & Wilkinson, 1966; Dunne *et al.*, 1986) with replicates of the precipitin line given by serum 942T in immunodiffusion with *S. mansoni* worm antigens (Fig. 1c). All rabbits were exsanguinated by cardiac puncture, and after separation the sera were stored at $-20°$.

Passive transfer of rabbit sera to infected mice

On each of Days 35, 36, 37 and 38 after infection with *S. mansoni*, mice in Groups c and d were injected intravenously with 0·5 ml rabbit serum that had been heated at 56° for 30 min and absorbed for 4 hr at room temperature with an equal volume of washed packed mouse erythrocytes.

Immunoelectrophoresis and immunodiffusion

Antigens released by packed *S. mansoni* worms gently agitated for 3 hr at room temperature in an equal volume of 2% deoxycholate in isotonic saline (protein concentration = 10 mg/ml, bovine serum albumin equivalent) were electrophoresed in agar and reacted with rabbit antisera as described previously (Dunne *et al.*, 1986). For immunodiffusion (Ouchterlony, 1958) the antigen well contained a tissue culture medium 199 extract of massed intact worms. Results of immunodiffusion and immunoelectrophoresis were recorded photographically over indirect light.

Zymography of immunoprecipitated antigens

Immunoelectrophoresis plates were washed for 24 hr at room temperature in excess isotonic saline and immersed for 1 hr at 37° in a solution containing 10 mg beta-naphthyl acetate and 10 mg Fast Blue B in 30 ml phosphate-buffered saline, pH 7·2, for detection of immunoprecipitated esterase activity (Uriel, 1963).

Immunoblot analysis of S. mansoni *worm antigens with rabbit antisera*

Antigens released by *S. mansoni* worms in 2% deoxycholate were resolved by electrophoresis in 8–20% SDS–PAGE (Studier, 1973), electroblotted on nitrocellulose paper and immunoenzymatically detected with 1:200 dilutions of rabbit antisera as described previously (Dunne *et al.*, 1986; Tsang *et al.*, 1983).

Indirect immunofluorescence with rabbit antisera

S. mansoni worms were perfused from mice treated 1 hr previously with 150 mg/kg praziquantel or from untreated mice. The parasites were rinsed in medium 199 warmed to 37°, incubated *in vitro* for 30 min at 37° with 1:40 dilution of rabbit antiserum, rinsed in fresh medium and incubated with 1:200 dilution of flouresceinated goat anti-rabbit IgG (Serotec, Oxford).

RESULTS

The results in Table 1, experiments 1 and 2, show that sera from two rabbits immunized respectively with whole homogenate of adult *S. mansoni* worms (1002M) and a detergent extract of worms (1018S) failed significantly to enhance the schistosomicidal activity of praziquantel. In contrast, rabbits 993S and 993T that had been immunized with culture medium extracts of adult worms yielded sera which did react synergistically with praziquantel in killing adult *S. mansoni* worms *in vivo* (Table 1, experiment 3).

Serum from a rabbit that had been repeatedly infected with cercariae via the ear (942T) also acted synergistically with praziquantel in *S. mansoni*-infected mice (Table 1, experiment 4). An anodally migrating antigen was precipitated in common by both the rabbit infection serum and the polyspecific sera after electrophoresis of detergent-extracted worm antigens in agar (Fig. 1a). On immersion of the plate in a chromogenic substrate mixture for non-specific esterases the precipitin line in question 'stained' violet (Fig. 1b).

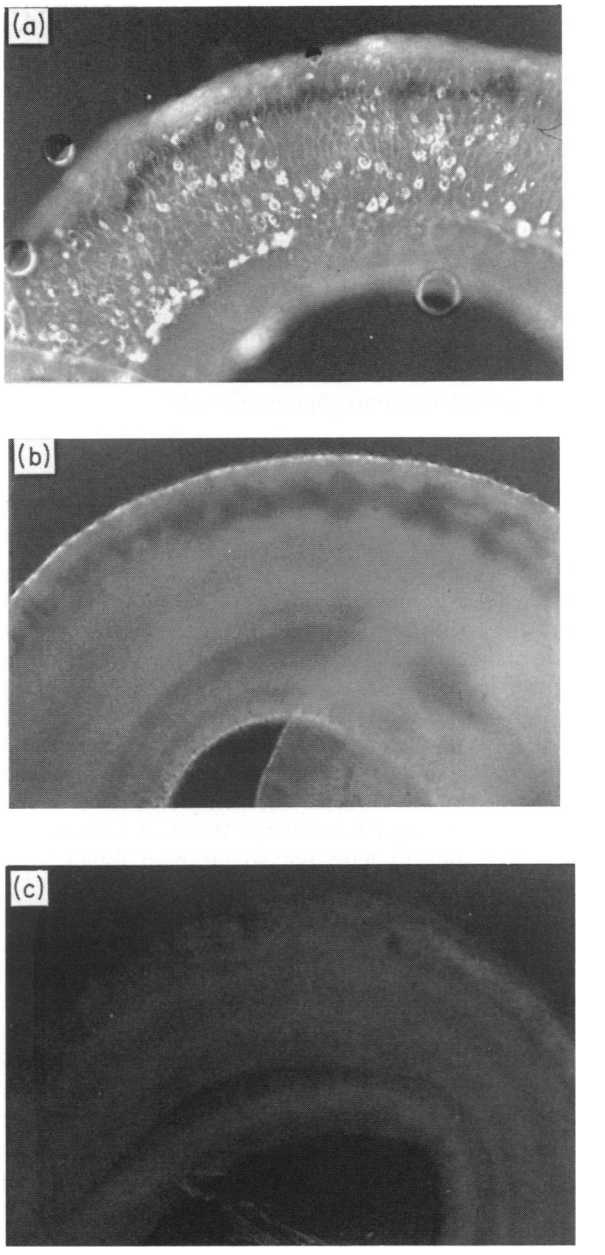

Figure 3. Indirect immunofluorescence. (a) Serum 1018J on worm from praziquantel-treated mouse. (The same pattern of fluorescence was obtained with 1018K). (b) 1018J on worm from untreated mouse. (c) Serum from rabbit immunized with complete Freund's adjuvant alone on worm from praziquantel-treated mouse.

Two further antisera (1018J and 1018K) were raised against the anodal antigen precipitated by the infection serum by immunizing rabbits with replicate immunodiffusion precipitin arcs. In immunodiffusion with antigens extracted by incubation of adult worms in tissue culture medium, the two resulting antisera were monospecific (Fig. 1c) and they reacted with the same antigen as the rabbit infection serum.

Both monospecific sera also reacted synergistically with praziquantel, with 1018K being particularly effective since it

increased the cure-rate from 33% worms killed by drug alone to 98% killed with drug plus the antiserum (Table 1, experiment 5).

No reductions in worm burdens occurred in separate experiments in which the same rabbit sera, both monospecific and polyspecific, were given to infected mice without drug.

The monospecific sera reacted predominantly against an antigen of approximately 27,000 MW molecular size in immunoblots (Fig. 2), the same antigen being a major reactant with the synergistically active polyspecific sera.

In indirect immunofluorescence the monospecific antisera reacted particularly with the tubercles of adult male worms from mice that had been treated orally with praziquantel 1 hr previously (Fig. 3a). The same sera stained the surface of worms from untreated mice only slightly (Fig. 3b).

DISCUSSION

The biochemical details of the schistosomicidal action of praziquantel are not well understood. However, the present results agree with previous evidence that the drug causes an erosion of the surface layers of the adult worm, particularly over the tubercles (Shaw & Erasmus, 1987), and an increased exposure of parasite-specific antigens (Harnet & Kusel, 1986). In addition to the esterase identified here by monospecific rabbit antisera, other antigens, including an alkaline phosphatase, were revealed on the worm surface after praziquantel treatment (J. Modha *et al.*, unpublished data). However, sera specific for these other antigens have not so far been found to react synergistically with praziquantel.

The physiological role of the esterase is not yet clear, but its concealment or disguise may be imperative for the parasite to survive in the immune host. The present observations are further evidence of the potential that enzymes might have as targets of anti-schistosome immunity (Smith *et al.*, 1986; Capron *et al.*, 1987; Tarrab Hazdai *et al.*, 1984). The form of the antigen recognized by the rabbit sera in culture medium extracts after immunodiffusion (Fig. 1c) may be different from that recognized in Western blots (Fig. 2), since the nearness of the immunoprecipitin line to the antigen well in Fig. 1c indicates a molecular size somewhat greater than that of immunoglobulins.

These results hint at the prospect of immunotherapy, not only for schistosomiasis, but also for other parasitic infections in which chemotherapy has been shown to be immune-dependent (Lwin *et al.*, 1987; Bianco *et al.*, 1986). Bitonti, McCann & Sjoerdsma (1986) have also shown that an antibody response is necessary in the treatment of *Trypanosoma brucei brucei* with alpha-difluoromethylornithine.

When identified and isolated, the antigens relevant to each of these systems might be modifiable so as to be capable of inducing immune responses which alone (i.e. in the absence of drug) can kill the parasite. As has been elegantly shown in the case of a transplantable lymphoma treated with asparaginase (Carter *et al.*, 1973), immune-dependent drug action also has implications for the onset of drug resistance.

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